

ON THE RATIONALE OF TREATING ALLERGIC DISEASES WITH BACTERIAL PYROGENS

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THE successful isolation of a pyrogenic polysaccharide from a *pseudomonas* species has stimulated a great deal of interest in its possible therapeutic applications.

The compound, obtained through tryptic digestion from cultures of the organism, has been sufficiently identified by chemical analysis to permit the preparation of solutions of uniform potency.¹ It has been offered to the medical profession under the name of Pyromen by Baxter Laboratories, Morton Grove, Illinois. Its activity can be measured either by induction of fever or by a rise in the number of circulating leukocytes following intravenous administration. The toxicity of the compound is low. The sensitivity of different experimental animals to its various effects differs widely.²

In recent years attention has been focused on the mediation of nonspecific effects of infection. The role of the hypothalamus, the anterior pituitary, and the adrenal cortex has been studied by numerous investigators. ACTH and adrenal corticosteroids produce some changes which are similar to those seen after infection, such as an increase in the number of circulating neutrophils, a depression of the number of circulating eosinophils; yet, while infection encourages fever, fever is abolished by ACTH and adrenal corticosteroids. In other words, the similarity between infection on one hand, and administration of ACTH and adrenal corticosteroids on the other, is limited. In fact, Soy-lemezoglu and Wells³ showed clearly that, in dogs, “. . . pyrogen produces its characteristic changes in the W.B.C. by mechanisms other than pituitary-adrenal discharge.”

In view of the changes in the circulating white blood cells produced by Pyromen, it seemed natural that many of the early investigators spoke about an “ACTH-like effect” of the compound.^{4, 5, 6} Consequently, it has been recommended for therapeutic use in a large number of clinical conditions known to respond to the administration of ACTH and adrenal corticosteroids. It is the purpose of this review to summarize experimental and clinical evidence for the usefulness of Pyromen in allergic diseases.

Allergic diseases consist of two well-distinguished phases: (1) a latent immunologic phase which includes exposure to an antigen followed by development and distribution of antibodies, and (2) a manifest “clinical” phase characterized by symptoms which are the result of antigen-antibody union in “shock tissues.” The nature of shock tissue has never been adequately explained; not all tissues which carry antibodies are shock tissues. In ragweed sensitivity of man, for instance, reagins are commonly found in the skin, yet only rarely does the skin participate in clinical manifestations of ragweed sensitivity.

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Increasing evidence suggests that the ground substance of a sensitized tissue plays an important role in determining extent and severity of clinical symptoms.⁷ ACTH and adrenal corticoids might exert their beneficial effect by restoring the normal physical and chemical function of impaired ground substance.

It can thus be readily seen that a polysaccharide such as Pyromen might affect allergic diseases in several ways. It might be antigenic in itself. It might alter antibody formation and distribution of antibodies. It might act on shock tissues either by stimulation of existing pituitary and adrenal regulations, or by direct effect on structures involved in the allergic reaction. During the past two years we have studied the effect of Pyromen on various phases of the allergic reaction. On the basis of our findings in guinea pigs it seemed necessary to re-examine the clinical effectiveness of the compound in patients suffering from allergic diseases.

EXPERIMENTAL PROCEDURE

A. Studies on Experimental Animals.—The details of our studies on guinea pigs will be reported elsewhere.⁸ In essence, the experiments were divided into four groups. The study of the first two groups (1 and 2) was designed to determine the effect of Pyromen on antibody formation; of the last two groups (3 and 4), to observe the effect of the polysaccharide on the behavior of the bronchial shock tissue following antigen-antibody union.

1. Pyromen (0.2 μ g) was injected subcutaneously every other day in guinea pigs sensitized and re-injected with antigens of known high potency, i.e., egg white and ragweed pollen extract adsorbed on aluminum cream. Pyromen injections were initiated one week prior to the sensitizing dose of the antigen. Sensitizing doses of ragweed extract were given intramuscularly, of egg white intraperitoneally; shocking doses were given intracardially after an interval of twenty-one days. Anaphylactic symptoms and death following re-injection were recorded for Pyromen-treated animals and untreated controls.

2. Pyromen (0.2 μ g) was injected subcutaneously every other day in guinea pigs sensitized and re-injected with a weak antigen of known potency, i.e., ragweed pollen extract not adsorbed on aluminum cream. In all other respects, the second group was treated like the first group.

3. A single dose of Pyromen (1.0 μ g) was given subcutaneously to guinea pigs sensitized with a strong antigen of known potency, i.e., egg white, four hours prior to intracardial re-injection of the antigen. Anaphylactic symptoms and death following re-injection were recorded for Pyromen-treated animals and untreated controls.

4. Nonsensitized guinea pigs, sensitized guinea pigs and guinea pigs which had survived anaphylactic shock, 24 hours previously, through protection with antihistamine drugs, were exposed to Pyromen by inhalation (8 μ g nebulized in a DeVilbiss No. 40 nebulizer over a period of 60 minutes). Nonsensitized guinea pigs and guinea pigs which had survived anaphylactic shock were sacrificed four hours after the end of Pyromen inhalation. Sensitized guinea pigs were re-injected intracardially after protection with antihistamine drugs 30 minutes after the end of the inhalation of Pyromen. They were sacrificed

24 hours later. Death of all animals of this group was produced by intraperitoneal injection of Nembutal. Lungs were removed immediately after death and prepared for histologic study.

Determinations of the number of circulating leukocytes and eosinophils were carried out on all four groups immediately prior to sensitization and to the re-injection of the homologous antigen; in surviving animals 24 and 48 hours after anaphylactic shock. In animals of Groups 1 and 2 total white blood cell counts and eosinophil counts were done, in addition, three times before and four hours after administration of Pyromen during the period of sensitization. In animals of Group 3 total white counts and eosinophil counts were done before and four hours after administration of Pyromen, immediately preceding re-injection of the homologous antigen; in Group 4 immediately after and four hours after inhalation of Pyromen.

*B. Clinical Studies.**—The clinical studies were divided into three groups: (1) a study to determine the antigenicity of Pyromen; (2) a study of the effect of Pyromen on the skin reactivity of ragweed-sensitive patients; and (3) a study of the clinical effectiveness of Pyromen in the treatment of allergic disorders of the anaphylactic type, i.e., rhinitis, bronchial asthma, and urticaria.

1. Studies to determine the antigenicity of Pyromen.

Fifty-four patients with various allergic disorders participated in this study. At the beginning of the survey each of the patients was tested by the intradermal injection of approximately 1/100 of a milliliter of a solution containing 10 micrograms of Pyromen per milliliter. Fifty-two of the patients gave negative tests; two patients showed a marked primary reaction—redness and swelling which failed to subside within 60 minutes. The remaining 52 patients were divided into subgroups of 38 and 14 patients each. Thirty-eight patients were given 0.3 c.c. of a solution containing 10 micrograms of Pyromen per milliliter subcutaneously every other day for a period of thirty days. Intradermal tests, using the same solution, were carried out at the end of this period. Fourteen patients were given one single injection of 0.3 c.c. solution containing 10 micrograms of Pyromen per milliliter and re-tested intradermally after an untreated interval of 21 days.

2. Study of the effect of Pyromen on skin tests.

Twelve ragweed-sensitive patients were selected for titration of skin sensitivity before and after administration of 0.3 c.c. of a solution containing 10 micrograms of Pyromen per milliliter subcutaneously every other day for 30 days. Intradermal tests were carried out before treatment and within one hour after the last Pyromen administration in accordance with the procedure outlined by Rappaport and co-workers:⁹

A freshly prepared extract of giant and short ragweed pollens which contained 50 per cent glycerol for preservation was diluted with normal saline solution for intradermal tests. The first dilution for skin tests contained 0.001 mg. total nitrogen per milliliter. This was diluted at tenfold intervals so that the final or

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sixth dilution was at a concentration of 0.00000001 mg. total N. per milliliter. The 6 dilutions and buffered normal saline solution which served as control were injected intradermally in 2 rows on the volar surface of the forearm. Special care was exercised in attempting to raise uniform wheals about 2 mm. in diameter. Ten minutes after the injection the wheals were examined, outlined with India ink, and transferred to Scotch tape for permanent recording. . . . The wheal sizes and the end points of the reactions recorded on the 2 days were compared in each patient.

3. Forty patients who participated in the clinical evaluation of the effectiveness of Pyromen were selected by the attending staff of the Allergy Clinic of the University of Illinois Research and Educational Hospitals. Of these, 19 had rhinitis, 13 bronchial asthma, and 8 urticaria.

Each of the patients had symptoms of moderate severity—none was intractable; physical examination and allergic survey had been completed prior to the onset of Pyromen therapy. With two exceptions routine treatment was discontinued while Pyromen was administered; symptomatic medication was reduced to a minimum compatible with the comfort of each individual.

Baxter Laboratories prepared Pyromen and Pyromen-Placebo in identical vials distinguished by number. Twenty patients were treated with vials containing Pyromen; twenty with the Pyromen solvent, a solution of 1/6 molar sodium r-lactate. The identity of each vial was kept secret and known only to Baxter Laboratories. It was not made known to us until after completion of the study.

Each patient was given 0.3 c.c. of a solution containing either 10 micrograms of Pyromen per milliliter or of Pyromen-Placebo subcutaneously for a period of 30 days. Name, clinic number, diagnosis, the number of the vial, a brief summary of the clinical response and a final evaluation classified as "improved," "unchanged," or "worse," were entered on cards by the physician in charge of the patient. The study was conducted from mid-March until the end of April, 1952. At the end of the experiment Dr. Robert Herwick, Medical Director of Baxter Laboratories, and one of us (M. S.) met and exchanged information about the contents of the vials and the recorded clinical results.

RESULTS

A. Experimental Studies.—Since we plan to publish the data of our studies on guinea pigs in detail, we would like to present here only a summary of the essential findings:

1. Administration of Pyromen prior to and during sensitization of guinea pigs to a strong antigen fails to decrease the incidence of anaphylactic shock upon re-injection of the antigen.

2. Administration of Pyromen prior to and during sensitization of guinea pigs to a weak antigen appears to increase the incidence of anaphylactic shock upon re-injection of the antigen. Statistical analysis suggests, however, that the number of experimental animals must be increased before valid conclusions can be drawn.

3. Administration of a single large dose of Pyromen four hours prior to re-injection of a potent antigen in highly sensitive guinea pigs fails to reduce

the incidence of anaphylactic shock. Each of the animals so treated showed a marked increase in bleeding tendency four hours after administration of the polysaccharide.

4. (a) Inhalation of Pyromen produces a marked neutrophilic infiltration of the bronchi of normal guinea pigs, with a corresponding decrease of circulating neutrophils. (b) Inhalation of Pyromen by guinea pigs which had a pre-existing bronchial eosinophilia produces a histological picture simulating Loeffler's Syndrome—a marked interstitial pneumonitis. Marked edema prevails; the interstitial spaces are filled with both neutrophils and eosinophils. (c) The inhalation of Pyromen by strongly sensitive guinea pigs 24 hours prior to re-injection of the homologous antigen does not prevent the immigration of eosinophils into the bronchial tissue which occurs as a rule within 24 hours after anaphylactic shock.

B. Clinical Studies.—1. Of 54 patients injected with Pyromen in repeated doses (three times a week, for 30 days) or once (three weeks prior to the test), none developed an anaphylactic sensitivity to the compound. On the other hand, two patients showed a strong primary reaction to the initial injection of the solution. The reaction was found to persist on subsequent injections; consequently, the patients were withdrawn from the experiment.

2. The administration of Pyromen does not alter the skin sensitivity of ragweed-sensitive patients.

3. Of 40 patients treated with Pyromen or Pyromen-Placebo for thirty days, only 38 completed the series of injections. One patient had to be admitted to the hospital because of a miscarriage; one patient had to leave the city unexpectedly. Of 19 patients treated with Pyromen, 8 improved, 7 showed no change, and 4 became worse. Of 19 patients treated with Pyromen-Placebo, 6 were improved, 9 showed no change, and 4 became worse. Of side effects, two patients treated with Pyromen complained about severe headaches without temperature elevation shortly after administration of the compound. The same side reaction was observed in three of the Placebo-treated patients. With this exception, the injections of Pyromen and Pyromen-Placebo were tolerated without untoward reactions.

DISCUSSION

A. Studies on Experimental Animals.—Guinea pigs have been selected for the experimental studies because they respond to sensitization and re-injection with anaphylactic antigens with well-defined and, under optimal conditions, uniform reactions; furthermore, we have used guinea pigs in various studies throughout the past five years and are thus familiar with the inherent variability of the species. Our findings in Groups 1 and 2 of our experimental animals indicate that Pyromen has no demonstrable inhibiting effect on formation and distribution of anaphylactic antibodies; in fact, they furnish suggestive evidence that Pyromen might facilitate sensitization to a weak antigen. In this respect our results are compatible with Stuart's observation that Pyromen, in contrast to cortisone, enhances the Arthus phenomenon in rabbits.¹⁰

The dose of Pyromen (1.0 μ g) administered to highly sensitive guinea pigs four hours prior to re-injection of the homologous antigen is very large. It

was very well tolerated but the animals showed a marked increase in bleeding four hours later when blood was obtained from the ear veins for total white cell counts and eosinophil counts.*

We hesitate to interpret the failure of Pyromen to diminish the incidence of anaphylactic shock as a failure of the compound to act on ground substance of the bronchial mucous membrane, particularly since Friedlaender¹² and co-workers have shown that ACTH administered four hours prior to re-injection of the homologous antigen equally fails to afford guinea pigs protection against anaphylactic shock.

The immigration of polymorphonuclear neutrophils into normal and anaphylactic "eosinophilic" bronchial tissue is compatible with our concepts of the effect of pyrogens. It is interesting, on the other hand, that eosinophilia develops subsequently to anaphylactic shock in bronchial tissue of guinea pigs which have been exposed to the inhalation of Pyromen. Since infection and allergic reaction are, as a rule, mutually exclusive, our findings seem to indicate that the neutrophilia produced by Pyromen is of short duration and can be superseded by the allergic stimulus. The sequence of the changes in the number of circulating leukocytes and eosinophils subsequent to the inhalation of Pyromen makes it likely that they are secondary in nature, mediated through tissue demand and not through pituitary-adrenal mechanisms.

Observations on guinea pigs cannot be applied to man without reservation. We¹³ have shown that guinea pigs respond only sluggishly to ACTH and corticoids and that inordinately large doses are required to accomplish physiologic changes comparable to those produced by comparatively much smaller doses in man.

B. Clinical Studies.—1. Observations on 54 patients who received Pyromen subcutaneously, either in one single dose or in repeated doses, made it obvious that the compound must be of low antigenicity or none to man. The primary reaction to the injection of the compound, which occurred in two patients, was persistent; we were able to reproduce the phenomenon on several subsequent occasions. We suspect that the reaction is caused by the solvent rather than by the polysaccharide, but we have not pursued the problem any further.

2. Pyromen does not alter the skin reactivity of ragweed-sensitive patients. Again this observation is of theoretical rather than practical interest, since ACTH fails to alter the skin reactivity even in patients who respond to its administration with considerable relief from clinical symptoms.

3. The evaluation of the effectiveness of drugs in the treatment of allergic manifestations is a difficult task. Allergic diseases have a functional component which often equals or even exceeds the structural changes caused by antigen-antibody union. In extensive tests of the effectiveness of antiasthmatic agents, it was found that patients suffering from severe respiratory disease responded to the administration of placebos of familiar antiasthmatic drugs with marked subjective improvement, even in the absence of objective changes of ventilatory function.¹⁴ It seemed likely that some of the reports on the effectiveness of

* It is interesting that Fitch and Washburne¹¹ mention "a tendency to increased bleeding on wounding" which occurred in several patients during Pyromen therapy.

Pyromen in the treatment of allergic diseases had failed to recognize the fact that treatment with frequent injections constitutes in itself a therapeutic procedure in patients with predominantly functional changes.

The clinical response of patients suffering from allergic disease of the anaphylactic type (namely, rhinitis, bronchial asthma, and urticaria) to the administration of Pyromen was disappointing. Improvement where reported was not spectacular; in the majority of patients objective tests failed to substantiate subjective claims. The ratio of patients who had improved to those who failed to respond or showed an aggravation of symptoms was almost identical to the ratio produced by the injection of Pyromen-Placebo during the same period. On the basis of our findings, it seems unlikely that Pyromen in subfebrile doses has a rationale in the treatment of allergic disease of the anaphylactic type. We have selected the term "in subfebrile doses" deliberately because it seems possible that Pyromen-produced fever will be of benefit to patients suffering from the limited number of allergic diseases which respond to other types of fever therapy. It is true that other investigators have reported satisfactory results upon the administration of smaller doses of Pyromen than those which have been given to our patients. We are unable to explain this discrepancy.

While intravenous injections of Pyromen, like any intravenous injections, might involve a certain risk, it is our impression that Pyromen can be safely given subcutaneously in the dosage which we have used. Side reactions are few; of those which we have seen, headaches observed in two patients were the outstanding complaint. Interestingly, three patients who received placebo suffered from headaches which were at least as pronounced as those observed in patients who received Pyromen. We do not understand the reason for this peculiar finding, but suspect that an idiosyncrasy to the solvent (1/6 molar sodium r-lactate) rather than to the polysaccharide might be responsible.

SUMMARY

1. Studies of the effect of Pyromen on sensitization and re-injection of guinea pigs with anaphylactic antigens failed to indicate that the compound produces a demonstrable decrease in formation of antibodies or in the results of antigen-antibody reactions.

2. The administration of Pyromen to 38 patients with allergic disease of the anaphylactic type (rhinitis, bronchial asthma, and urticaria) failed to indicate that the clinical effectiveness of the compound is superior to a placebo containing its solvent, 1/6 molar sodium r-lactate.

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